EXHIBIT E

MEDLINE(R)

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Tumor necrosis factor alpha induces apoptosis in mammary adenocarcinoma cells by an increase in intranuclear free Ca2+ concentration and DNA fragmentation.

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The incubation of human mammary adenocarcinoma cells (BT-20) with tumor necrosis factor alpha in the absence or presence of cycloheximide resulted in progressive DNA fragmentation. This was preceded by a sustained increase in intracellular free Ca2+ concentration and was not detected in cells pretreated with intracellular Ca2+ chelators, calmodulin antagonists, or activators of protein kinase C. Image analysis of fura-2-loaded BT-20 cells treated with tumor necrosis factor alpha revealed that, in many cells, the initial increase in Ca2+ level occurred in a cellular region that corresponded to the localization of the nucleus. Our findings suggest that tumor necrosis factor alpha can promote an increase in intranuclear free Ca2+ which, in turn, may stimulate Ca(2+)-dependent endonuclease activity, resulting in DNA fragmentation and apoptosis.

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Tumour necrosis factor-alpha increases intracellular Ca2+ and induces a depolarization in cultured astroglial cells.

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Tumour necrosis factor (TNF)-alpha, a strong immune mediator, is released within the brain during inflammatory diseases and contributes to immunological activation of glial cells. Here we report that, in astrocytes, TNF-alpha also affects the intracellular Ca2+ homeostasis and basic electrophysiological properties such as the membrane potential. Using the Ca2+ indicator dye fura-2 in a cell culture model, we found that TNF-alpha (10-1000 U ml-1), but not interleukin 1 or 6, induced a slow but more than two-fold increase of the intracellular Ca2+ concentration, which could be blocked by Co2+ (1.0 mM), vèrapamil (100 microM) or omission of external Ca2+. This intracellular Ca2+ increase was accompanied by a marked decrease of the membrane potential by 35 mV. CSF of patients with bacterial meningitis, known to contain large amounts of TNF-alpha, induced a similar depolarization of astrocytes, which was markedly reduced by a neutralizing anti-TNF-alpha antibody. We conclude that TNF-alpha induces an increase of intracellular Ca2+ and a depolarization in astrocytes with the consequence of disturbing voltage-dependent glial functions such as regulation of local ion concentrations and glutamate uptake. During inflammatory CNS diseases this immuno-electrical coupling may contribute to an impairment of neuronal function.